Design and Synthesis of Novel Carvedilol Analogues that Suppress Store-Overload-Induced-Ca\(^{2+}\) Release

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ABSTRACT

The Store-Overload-Induced-Ca\(^{2+}\) -release (SOICR) through a defective RyR2 channel triggers delayed afterdepolarizations (DADs) that can lead to ventricular tachyarrhythmias and sudden death in individual with heart failure. We tested many \(\beta\) blockers for SOICR inhibition and found that carvedilol is the only beta blockers that suppress SOICR. Strong SOICR inhibition required high dose of carvedilol which would lead to excessive \(\beta\) - blocking and causes bradycardia . We designed and synthesized many analogs of Carvedilol for minimal beta blocking activity as potential drugs for Ca\(^{2+}\) mediated cardiac arrhythmias. We did both \textit{in vitro} and \textit{in vivo} (mice & rabbit) bioassay of those synthesized analogs. Parent Carvedilol displayed an IC\(_{50}\) of 15.9 \(\mu\)M and many compounds were found to be better inhibitors. The clinical trials of those compounds are under progress. If we get fruitful result, one of the synthesized compounds will be the potential drug for Ca mediated cardiac arrhythmias.

Key Words: SOICR, ventricular tachyarrhythmias, Carvedilol, inhibitor, IC\(_{50}\), analogues

INTRODUCTION

Ventricular arrhythmias are a leading cause of sudden death, particularly in patients with heart failure. Consequently, a variety of antiarrhythmic drug therapies have been evaluated in clinical trials, which revealed only limited survival benefits.\(^1,2\) Antagonists of \(\beta\)-adrenergic receptors (\(\beta\)-blockers) have been of special interest in these studies, as overstimulation of these receptors can trigger fatal ventricular arrhythmias.\(^3,4\) The underlying mechanism of this process involves, in part, an overload of Ca\(^{2+}\) in the sarcoplasmic reticulum, which results in spontaneous Ca\(^{2+}\) efflux through the RyR2 Ca\(^{2+}\)-release channel.\(^5\) In turn, this store-overload-induced calcium release (SOICR) through a defective RyR2\(^{6,7}\) triggers delayed afterdepolarizations (DADs),\(^8,9\) which have

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been implicated in catecholaminergic polymorphic ventricular tachycardias (CPVTs) as well as in ventricular tachyarrhythmias and sudden death.\textsuperscript{3,4}

More recently, we demonstrated that a variety of other \(\alpha\) - and \(\beta\) -blockers, except the nonselective \(\beta\)-blocker carvedilol, failed in the suppression of SOICR.\textsuperscript{10} Unfortunately, the benefits of carvedilol therapy are limited by drug intolerance and excessive \(\beta\)-blockade, with attendant complications of bradycardia and hypotension.\textsuperscript{1} This suggests that the unique efficacy of carvedilol in suppressing SOICR occurs independently of its \(\alpha\) - and \(\beta\)-blocking activity and its antioxidant properties, and it is instead principally because of its ability to stabilize \(\text{Ca}^{2+}\) handling via the RyR2 channel. Indeed, we designed and synthesized some novel carvedilol analogues with comparable abilities to inhibit SOICR to that of the parent compound carvedilol 3a (ca. 10 \(\mu\)M) but with strongly attenuated \(\beta\)-blockade (ca. micromolar compared to nanomolar for 3a). Many compounds proved highly effective in preventing stress-induced ventricular arrhythmias in mice (vide infra) without the undesired effects of excessive \(\beta\)-blockade.\textsuperscript{10} For the purpose of these studies, a convenient single-cell bioassay was developed for measuring SOICR suppression by drug candidates, which is based on human embryonic kidney (HEK 293) cells expressing a mutant RyR2 channel (R4496C).\textsuperscript{5} This mutation results in spontaneous calcium release from the endoplasmic reticulum of the cells through the defective channel, with calcium efflux detected by the measurement of fluorescence from the \(\text{Ca}^{2+}\)-sensitive indicator dye fura 2/AM.

\begin{equation}
\text{Scheme 1}
\end{equation}

\begin{align}
\text{1} + \text{H}_2\text{N}\text{R} & \xrightarrow{a} \text{3a-d} \\
3a-d & \xrightarrow{b, c} 4a-d \\
4a-d & \xrightarrow{d} 5a-d \\
5a-d & \xrightarrow{e} 6a-d
\end{align}

\begin{align}
R &= \text{a) LiBr, DME, 50\degree C or i-PrOH, reflux (b) Chloroacetyl chloride, Et}_3\text{N, CHCl}_3, \text{RT (c) NaH, THF, RT (e) CDI, Et}_3\text{N, CH}_2\text{Cl}_2, 0\degree \text{C \cdot RT}}
\end{align}

**EXPERIMENTAL**

All compounds subjected to bioassay for which elemental analyses were not provided were of >95\% purity as determined by HPLC analysis employing the following conditions: Novapak C\textsubscript{18}


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reversed-phase column, 3.9 × 150 mm; solvent: acetonitrile/water, 70:30, 0.8 mL/min; UV detector: 254 nm. \(^1\)H NMR spectra were obtained at 300 or 400 MHz. \(^{13}\)C NMR spectra were obtained at 75 or 101 MHz.

The synthesis of compounds 3a-d was achieved by reacting the commercially available epoxides 1 with the corresponding amines 2a-d, as shown in Scheme 1. Cyclization of amino alcohols 3a-d with chloroacetyl chloride afforded lactams 4a-d, whereas the use of 1,1'-carbonyldiimidazole afforded products 6a-d. The reduction of 4a-d with lithium aluminum hydride provided the corresponding 5a-d. The methylated product 7 was prepared by methylation of 3a with iodomethane, whereas 10 was obtained from 3a by cyclization and N-methylation with dimethyl carbonate in one step followed by hydrolysis (Scheme 2). Compound 8 was produced from 3a by methylation using excess iodomethane. Product VKII-86 was prepared from the reaction of amine 2a with the homologated epoxides 11. Compound 13 was obtained from epoxide 1 and amine 12 as summarized in Scheme 3.

RESULT AND DISCUSSION
Carvedilol is the only beta blocker that suppresses SOICR

To determine whether carvedilol possesses a unique action on intra-cellular Ca\(^{2+}\) handling, we assessed the impact of a number of beta blockers on SOICR in HEK293 cells expressing a SOICR-promoting, CPVT-causing RyR2 mutation (R4496C). Of the 14 beta blockers tested, only carvedilol 3a effectively inhibited SOICR (>95%). All the other beta blockers tested had no impact on SOICR even at relatively high concentrations (30 \(\mu\)M; Fig. 1). The benchmark compound 3a displayed an IC\(_{50}\) of 15.9 \(\mu\)M (Table 1, entry 1) in this assay. We first prepared and assayed several derivatives (3b - d) with modified catechol groups. Aromatic hydroxylation of the catechol moiety of carvedilol at the 4′- and 5′-positions is known to afford phenolic metabolites.\(^{11}\) Modification of these sites to probe their effect on SOICR inhibition was therefore of particular interest (entries 2–4). The introduction of 4′- and 5′-chloro substituents in 3b slightly improved SOICR activity, but replacement of the methoxy group with a third chlorine atom in 3c diminished the potency significantly.

![Figure 1 Carvedilol inhibits SOICR in HEK293 cells](image)

We also investigated whether SOICR inhibition would be affected by manipulation of the \(\beta\)-amino alcohol moiety in the linker chain, which plays a critical role in the excessive \(\beta\)-blockade encountered with carvedilol. Entries in Table 1 show the IC\(_{50}\) values for analogues where this functionality was incorporated into various rings with or without the previous modifications to the catechol region of the carvedilol molecule given in Table 1. In the case of \(\delta\)-lactams 4a-d, there was a moderate loss of activity compared to 3a when either the intact catechol moiety was retained (4a) or when it was replaced by a simple cyclohexyl group (4d). Moreover, the chlorinated derivatives 4b and 4c were essentially devoid of activity when introduced together with lactamization. The morpholine analogues 5a-d revealed no consistent pattern of behavior when compared with the corresponding free \(\beta\)-amino alcohols 3a-d and with the corresponding lactams 4a-d, respectively. Although a slight decrease in activity was observed in 4a and 5a compared to 3a, the chlorinated analogues 5b and 5c were intermediate between the corresponding amino alcohols 3a and 3b and the inactive lactams 4b and 4c. The cyclohexyl derivative 5d was devoid of activity, in contrast to the weakly active amino alcohol 3d. The cyclic carbamates 6a-d all showed poor SOICR inhibition compared to the corresponding amino alcohols 3a-d. Furthermore, alkylation of the aliphatic secondary amino group in 7 had little effect, whereas alkylation of the carbazole nitrogen in 10 resulted in diminished activity. Surprisingly, exhaustive alkylation of both nitrogens and of the secondary alcohol group in 8 produced a more strongly SOICR-inhibiting compound than either 3a, 7, or 10.

These results demonstrate that considerable variation in the structure of carvedilol is possible while retaining strong SOICR-suppressing activity in the RyR2-R4496C mutant HEK293 single cell assay. The highly potent analogues of carvedilol reveal that significant changes can be tolerated in the catechol and linker without loss of activity relative to the clinically useful drug 3a.
All of the compounds in Table 1, where modifications to the catechol subunit are listed, show significant activity. Beneficial catechol modifications include chlorination of the 4′- and 5′-positions (compound 3b), which is expected to block metabolic oxidation at those sites and possibly retard clearance. This suggests that the compounds in Table 1 may prove good candidates as SOICR inhibitors.

The β-amino alcohol functionality plays a key role in mediating β-adrenergic blockade via multiple hydrogen-bonding interactions with the β-receptor. In order to determine whether or not this functionality plays a similar role in SOICR inhibition, we investigated the alkylation of these key groups via incorporation into cyclic structures or by simple methylation. However, Table 1 indicates mixed results with respect to the SOICR inhibition shown by such compounds. Although the lactam and morpholine derivatives of carvedilol (4a and 5a, respectively), as well as the O- and N-methylated compounds 7-10 showed similar or slightly lower SOICR inhibition compared to 3a; further alteration of these structures was generally accompanied by severe or total loss of activity. The homologated analogues VKII-86 and 13 showed strong SOICR suppression in mutant HEK 293 cells.12

Table 1. SOICR Inhibition by synthesized Analogues

<table>
<thead>
<tr>
<th>S. No.</th>
<th>Compound</th>
<th>IC₅₀ (µM)</th>
<th>Repeats (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3a (Carvedilol)</td>
<td>15.9 ± 2.5</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>3b</td>
<td>11.2±1.2</td>
<td>3</td>
</tr>
<tr>
<td>3</td>
<td>3c</td>
<td>28.0 ±0.6</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>3d</td>
<td>35.2 ±2.2</td>
<td>4</td>
</tr>
<tr>
<td>5</td>
<td>4a</td>
<td>26.1 ±3.6</td>
<td>14</td>
</tr>
<tr>
<td>6</td>
<td>4b</td>
<td>&gt;1000</td>
<td>3</td>
</tr>
<tr>
<td>7</td>
<td>4c</td>
<td>&gt;1000</td>
<td>3</td>
</tr>
<tr>
<td>8</td>
<td>4d</td>
<td>52.9±15</td>
<td>4</td>
</tr>
<tr>
<td>9</td>
<td>4e</td>
<td>22.9±2.7</td>
<td>3</td>
</tr>
<tr>
<td>10</td>
<td>5a</td>
<td>86.7±36</td>
<td>3</td>
</tr>
<tr>
<td>11</td>
<td>5b</td>
<td>364±52</td>
<td>3</td>
</tr>
<tr>
<td>12</td>
<td>5c</td>
<td>&gt;1000</td>
<td>3</td>
</tr>
<tr>
<td>13</td>
<td>5d</td>
<td>86.3±52</td>
<td>3</td>
</tr>
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<td>14</td>
<td>6a</td>
<td>97.2±63</td>
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<td>15</td>
<td>6b</td>
<td>105±5</td>
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</tr>
<tr>
<td>16</td>
<td>6c</td>
<td>113±25</td>
<td>5</td>
</tr>
<tr>
<td>17</td>
<td>6d</td>
<td>18.3±0.0</td>
<td>4</td>
</tr>
<tr>
<td>18</td>
<td>8</td>
<td>11.7±0.7</td>
<td>3</td>
</tr>
<tr>
<td>19</td>
<td>10</td>
<td>35.2±5.2</td>
<td>3</td>
</tr>
<tr>
<td>20</td>
<td>VKII-86</td>
<td>16.8± 3.3</td>
<td>4</td>
</tr>
<tr>
<td>21</td>
<td>13</td>
<td>20.0±1.6</td>
<td>3</td>
</tr>
</tbody>
</table>

Combined SOICR inhibition and beta blockade

We used metoprolol or bisoprolol as a selective beta blocker and VK-II-86 as a SOICR inhibitor to test the premise that higher antiarrhythmic efficacy can be achieved by both SOICR inhibition and beta blockade compared to SOICR inhibition or beta blockade alone. Treatment of mice heterozygous for the RyR2 R4496C mutation with VK-II-86 plus metoprolol or with VK-II-86 plus bisoprolol was much more effective in preventing CPVT than was treatment with VK-II-86 alone.
These data show that the combination of SOICR inhibition and beta blockade increases antiarrhythmic efficacy.

**Figure 2**: Combined effects of VKII-86 and beta blocker on CPVT in R4496C – heterozygous or homozygous mice

### CONCLUSION

Carvedilol, with both beta-blocking and anti-SOICR activities, has the capability of SOICR by regulating calcium efflux through the RyR2 channel. However, the concentrations of carvedilol required to suppress SOICR (0.3–1 µM) are much higher than those required for beta blockade (~1 nM). Therefore, strong SOICR inhibition would require high doses of carvedilol, which could produce excessive beta blockade and accompanying adverse effects such as bradycardia. We designed and synthesized many analogues of carvedilol and screened them for their SOICR-suppressing effects on the RyR2-R4496C mutant HEK293 cell line. By synthesizing several carvedilol analogs with structural changes in regions thought to be essential for beta blockade, we pharmacologically separated the beta-blocking and anti-SOICR activities of carvedilol, so that the benefits of beta blocking and SOICR inhibition could be individually and optimally titrated. Some carvedilol analogs, like 3b, 8 and VK-II-86, exhibited minimal beta-blocking activity, but retained the ability to suppress SOICR. These new agents prevented CPVT in mice without causing bradycardia. We also showed that VK-II-86, when combined with selective beta blockers (metoprolol or bisoprolol), is more effective than VK-II-86, metoprolol or bisoprolol alone in suppressing ventricular tachyarrhythmias. Thus, SOICR inhibition combined with selective beta blockade represents a new and promising approach for better preventing Ca^{2+}-mediated cardiac arrhythmias.

### REFERENCES

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